

Risk factors of platelet refractoriness in children

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ABSTRACT

Therapeutic platelet transfusions are often performed in pediatric patients with various indications. However, the platelet transfusion has potentially induced more harm than good for some patients. Therefore, its effectiveness needs to be evaluated. This study aimed to evaluate the clinical risk factors namely sepsis, splenomegaly, DIC (disseminated intravascular coagulation), severe bleeding and the history of platelet transfusion in the incidence of platelet refractoriness. This was a case-control study conducted over a 13-month period from August 1st, 2010 until September 30th, 2011 in Department of Pediatrics, Dr. Sardjito General Hospital, Yogyakarta. From a total of 1403 platelet transfusion episodes in 464 patients, 86 incidences of refractoriness and 86 of nonrefractoriness were observed. Bivariate analysis showed that sepsis [OR = 5.91 (2.90-12.05); p = 0.000], splenomegaly [OR = 2.82 (1.32-6.04); p = 0.006], severe bleeding [OR = 8.41 (4.19-16.871); p = 0.000], DIC [OR = 22.96 (6.73-78.35); p = 0.000] and the history of platelet transfusions [OR = 5.33 (2.78-10.23); p = 0.000] increased the risk of platelets refractoriness. Furthermore, multivariate analysis showed that sepsis (OR = 2.96; 95%CI: 1.19-7.32; p = 0.019), splenomegaly (OR = 3.94; 95% CI: 2.21-16.00; p = 0.000), severe bleeding (OR = 3.53; 95% CI: 1.40-8.89; p = 0.008), DIC (OR = 5.54; 95% CI: 1.29-22.75; p = 0.021) and the history of platelet transfusion (OR = 2.84; 95% CI: 2.74-9.77; p = 0.001) were the independent risk factors for the occurrence of platelet refractoriness. In conclusion, sepsis, splenomegaly, severe bleeding, DIC, and the history of platelet transfusion are the risk factors of platelet refractoriness in pediatric patients.

ABSTRAK

Terapi transfusi trombosit sering dilakukan pada pasien anak dengan berbagai indikasi. Namun demikian transfusi trombosit mempunyai potensi menimbulkan bahaya dari pada manfaatnya. Oleh karena itu efektivitasnya perlu dievaluasi. Penelitian ini bertujuan untuk mengevaluasi faktor risiko klinik yaitu sepsis, splenomegali, DIC (*disseminated intravascular coagulation*), pendarahan berat dan riwayat transfusi trombosit pada kejadian refrakter trombosit. Penelitian ini merupakan penelitian kasus-kontrol yang dilakukan selama 13 bulan dari 1 Agustus 2010 sampai 30 September 2011 di Bagian Ilmu Kesehatan Anak, Rumah Sakit Umum Dr. Sardjito, Yogyakarta. Dari total 1403 episode transfusi trombosit pada 464 pasien, teramati 86 kejadian refrakter dan 86 tidak terjadi refrakter. Analisis bivariate menunjukkan bahwa sepsis [OR = 5,91 (2,90-12,05); p = 0,000], splenomegali [OR = 2,82 (1,32-6,04); p = 0,006], pendarahan berat [OR = 8,41 (4,19-16,871); p = 0,000], DIC [OR = 22,96 (6,73-78,35); p = 0,000] dan riwayat transfusi trombosit [OR = 5,33 (2,78-10,23); p = 0,000] meningkatkan risiko refrakter trombosit. Analisis mulivariat selanjutnya menunjukkan bahwa sepsis (OR = 2,96; 95%CI: 1,19-7,32; p = 0,019), splenomegali (OR = 3,94; 95% CI: 2,21-16,00; p = 0,000), pendarahan berat (OR = 3,53; 95% CI : 1,40-8,89; p = 0,008), DIC (OR = 5,54; 95% CI: 1,29-22,75; p = 0,021) dan riwayat transfusi trombosit (OR = 2,84; 95% CI: 2,74-9,77; p = 0.001) merupakan faktor risiko independen terjadinya refrakter trombosit. Dapat disimpulkan bahwa sepsis, splenomegali, pendarahan berat, DIC dan riwayat transfusi trombosit merupakan faktor risiko terjadinya refrakter trombosit pada pasien anak.

Keywords: refractory platelets - CCI - number of platelets – children

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INTRODUCTION

Platelets are well known to have a large variety of functions. Platelets are main regulators of hemostasis where they contribute to the hemostatic plug and accelerate the coagulation system.¹ Platelets also contribute to the pathogenesis of atherosclerosis and vascular inflammation,^{2,3} inflammatory-mediated diseases such as arthritis, systemic lupus and sepsis.⁴ Moreover, platelets are involved in innate and adaptive immunity as well as in cancer metastasis.⁵⁻⁷

Since platelet transfusions have been proven to be able to reduce mortality from haemorrhage in patients with acute leukaemia in the 1950s, the use of the platelet transfusion has steadily grown to become an essential part of the treatment of major haemorrhagic complications associated with the management of haematological and oncological disorders.^{8,9} Today, more than 1.5 million platelet products are transfused in the USA and 2.9 million platelet products in Europe annually.⁹

Therapeutic platelet transfusions are widely accepted as indicated in children with severe thrombocytopenia and/or platelet dysfunction associated with active serious bleeding.^{8,10} Children thrombocytopenia can be caused by a number of disease processes such as decreased production (leukemia, systemic infection), increased destruction (idiopathic thrombocytopenia purpura), medication-induced (chemotherapy drugs) and others.¹¹ The benefits of the therapeutic platelet transfusions can not be denied. However, due to increased concerns about their risks, such as transmission of various pathogens, fever, anaphylaxis and alloimmunization, the therapeutic platelet transfusion is being challenged as perhaps being without evidence base, and potentially inducing more harm than good for some patients.¹²

Therefore, the evaluation of the risk to benefits ratio of the platelets transfusions should be conducted. This study was conducted to evaluate risk factors of platelet refractoriness in pediatric patients who underwent platelet transfusions in Department of Pediatrics, Dr. Sardjito General Hospital, Yogyakarta.

MATERIALS AND METHODS

This was an observational study using case-control design aiming to evaluate sepsis, splenomegaly, severe bleeding, DIC (disseminated intravascular coagulation), and the history of platelet transfusion as the risk factors for platelet refractoriness in the children who underwent platelet transfusions and met the inclusion and exclusion criteria. The inclusion criteria were children aged 0-18 years, underwent platelet transfusion and thrombocyte assessment one hour or 16-24 hours or early morning of the next day after platelet transfusion. The exclusion criteria were children with ITP (immune thrombocytopenic purpura) who received amphotericin B or heparin.

The case group consisted of patients who underwent platelet transfusion and had refractoriness to the platelet transfusion, whereas the control group consisted of patients who underwent platelet transfusion but had no refractoriness. Patients were considered having the refractoriness if they had the CCI (corrected count increment) of $< 5,000/\mu\text{L}$ one hour after transfusion or $\hat{A}2,500/\mu\text{L}$ 16-24 hours or early morning of the next day after transfusion, whereas patients were considered having no refractoriness if they had the CCI of $\geq 5,000/\mu\text{L}$ one hour after transfusion or $\geq 2,500/\mu\text{L}$ 16-24 hours or early morning of the next day after transfusion.^{12,15} Sample size was calculated using the formula to test the hypothesis of unpaired analysis. Therefore, the minimal number of subject in each group needed in this study was 76.

Characteristics subjects, clinical data including sepsis, splenomegaly, severe bleeding, DIC, and the history of the platelet transfusion were obtained from the medical records of each patient of Department of Pediatrics, Dr. Sardjito General Hospital, Yogyakarta from August 1st, 2010 to September 30th, 2011. The study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Bivariate analysis using Chi-square (χ^2) was used to evaluate sepsis, splenomegaly, severe bleeding, DIC, and the history of the platelet transfusion as risk factors of platelet refractoriness. Furthermore, multivariate analysis using logistic regression analysis was used to evaluate the relationship between the categorical independent variables (sepsis, splenomegaly, severe bleeding, DIC, and the history of the platelet transfusion) and dichotomous dependent variable (platelet refractoriness). Odds ratio (OR) with 95% confidence intervals (95% CI) was applied to assess the risk of the platelet refractoriness between the group with risk factors and the group without risk factors. A p value of less than 0.05 was considered to be significant.

RESULTS

Over a 13-month period from August 1st, 2010 until September 30th, 2011, data were collected from a total of 1403 platelet transfusion episodes in 464 patients in Dr. Sardjito General Hospital. During the platelet transfusion episodes, 86 refractory incidences

and 86 non refractory incidences that met the criteria of study were identified. The refractory examination was based on CCI conducted 24 hours or early morning of the next day after transfusion.

The characteristics of patients who met the criteria of study are presented in TABLE 1. No significant difference was observed in the number of patients based on gender in the refractory group compared to the non refractory group ($p=0.645$). In addition, the number of patients based on age in the refractory group was also not significantly different compared to the non refractory group ($p=0.229$). However, all risk factors of platelet refractoriness namely sepsis, splenomegaly, severe bleeding, DIC, as well as the history of platelet transfusion observed in this study between refractory group and non refractory group were not significantly different ($p<0.05$). Moreover, a patient with more than one risk factors of platelet refractoriness in same time in both refractory and no refractory groups was observed in this study. Sepsis together with splenomegaly, DIC together and/or heavy bleeding and platelet transfusions history could be confirmed in a patient.

TABLE 2 shows the bivariate analysis of the relationship between the risk factors observed in this study and the occurrence of platelet refractoriness. Sepsis, splenomegaly, severe bleeding, DIC and the history of platelet transfusion significantly increased the occurrence of platelet refractoriness in pediatric patients who underwent platelet transfusion with different level ($p<0.05$).

TABLE 1. The characteristics of patients

Characteristics	Refractory (n=86)	Non refractory (n=86)	p
	n (%)	n (%)	
Gender			
• Male	50 (58.1)	47 (54.7)	0.645
• Female	36 (41.9)	39 (45.3)	
Age (years)			
• 0-5	57 (66.3)	52 (60.5)	0.229
• 5<10	21 (24.4)	17 (19.8)	
• >10	8 (9.3)	17 (19.8)	
Sepsis			
• Yes	72 (83.7)	40 (46.5)	0.000*
• No	14 (16.3)	46 (53.5)	
Splenomegaly			
• Yes	27 (31.4)	12 (14.0)	0.006*
• No	59 (68.6)	74 (86.0)	
Heavy bleeding			
• Yes	58 (67.4)	17 (19.8)	0.000*
• No	28 (32.6)	69 (80.2)	
DIC			
• Yes	39 (45.3)	3 (3.5)	0.000*
• No	47 (54.7)	83 (96.5)	
History of platelet transfusion			
• Yes	61 (70.9)	27 (31.4)	0.000*
• No	25 (29.1)	59 (68.6)	

DIC = disseminated intravascular coagulation; * Significantly different ($p < 0.05$)

TABLE 2. Bivariate analysis of the relationship between the risk factors and platelet refractoriness of pediatric patients who underwent platelet transfusion

Characteristics	Refractory (n=86)	Non refractory (n=86)	OR (95% CI)	p
	n (%)	n (%)		
Sepsis				
• Yes	72 (83.7)	40 (46.5)	5.91	0.000*
• No	14 (16.3)	46 (53.5)	(2.90-12.05)	
Splenomegaly				
• Yes	27 (31.4)	12 (14.0)	2.82	0.006*
• No	59 (68.6)	74 (86.0)	(1.31-6.04)	
Heavy bleeding				
• Yes	58 (67.4)	17 (19.8)	8.04	0.000*
• No	28 (32.6)	69 (80.2)	(4.19-16.87)	
DIC				
• Yes	39 (45.3)	3 (3.5)	22.95	0.000*
• No	47 (54.7)	83 (96.5)	(6.72-78.35)	
History of platelet transfusion				
• Yes	61 (70.9)	27 (31.4)	5.33	0.000*
• No	25 (29.1)	59 (68.6)	(2.78-10.22)	

*DIC = disseminated intravascular coagulation * Significantly different (p<0.05)*

TABLE 3 shows the multivariate analysis of the relationship between the risk factors observed in this study and the occurrence of platelet refractoriness. Sepsis, splenomegaly, severe bleeding, DIC and the history of platelet

transfusion were independent risk factors that increased the occurrence of platelet refractoriness in pediatric patients who underwent platelet transfusion with different level (p<0.05).

TABLE 3. Multivariate analysis of the relationship between the risk factors and platelet refractoriness of pediatric patients who underwent platelet transfusion

Characteristics	Refractory (n=86)	Non refractory (n=86)	OR (95% CI)	p
	n (%)	n (%)		
Sepsis				
• Yes	72 (83.7)	40 (46.5)	2.96	0.019*
• No	14 (16.3)	46 (53.5)	(1.19-7.32)	
Splénomegaly				
• Yes	27 (31.4)	12 (14.0)	3.94	0.000*
• No	59 (68.6)	74 (86.0)	(2.20-16.00)	
Heavy bleeding				
• Yes	58 (67.4)	17 (19.8)	3.53	0.008*
• No	28 (32.6)	69 (80.2)	(1.40-8.89)	
DIC				
• Yes	39 (45.3)	3 (3.5)	5.41	0.021*
• No	47 (54.7)	83 (96.5)	(1.29-6.31)	
History of platelet transfusion				
• Yes	61 (70.9)	27 (31.4)	2.84	0.010*
• No	25 (29.1)	59 (68.6)	(1.28-6.31)	

DIC = disseminated intravascular coagulation * Significantly different ($p < 0.05$)

DISCUSSION

Platelet refractoriness is a result of the shortened survival of platelets caused by factors of non-immunologic and/or immunologic origins. Non-immunologic causes involve sepsis, fever, splénomegaly, bone marrow and peripheral blood progenitor cell transplantation, disseminated intravascular coagulation, graft-versus-host disease, vaso-occlusive diseases, drug induced thrombocytopenia and hemorrhages.^{11,12} The immunologic causes involve antibodies against the ABO system, human leukocyte antigen (HLA) and/or human platelet antigen (HPA) present on the membrane of donor platelets.¹³⁻¹⁵

In this study, the causes of platelet refractoriness in pediatric patients who

underwent platelet transfusions were evaluated. Sepsis, splénomegaly, severe bleeding, DIC and the history of platelet transfusion have been proven as independent risk factors that increased the occurrence of platelet refractoriness in pediatric patients.

Sepsis increased the occurrence of platelet refractoriness 3 times higher (OR = 2.96) than in children without sepsis. These results are similar to studies conducted in adult patients that found the relative risk of platelet transfusion refractoriness was 2.73.¹¹ Platelet refractoriness in patients with sepsis occurs through the activation of prothrombin by cytokines.¹⁶ Lipopolysaccharides found in the outer membrane of bacteria acts as endotoxins and activates platelets directly.¹⁷ In addition,

hematophagocytosis has been reported as a cause of thrombocytopenia in sepsis, although its mechanisms remain unclear. It is thought that macrophage plays an important role in thrombocytopenia in sepsis. The macrophage-colony-stimulating factor (M-CSF) is over-produced in the sepsis syndrome, particularly when hemophagocytosis is present.¹⁸

Meanwhile, the risk of platelet refractoriness increased 4 times (OR = 3.94) in pediatric patients with splenomegaly in this study. Platelet refractoriness in patients with splenomegaly occurs due to the increase of platelet sequestration that increases proportionally with the enlargement of spleen.¹⁹ The risk of platelet refractoriness due to splenomegaly reported in this study was higher than another study conducted in adult patients which reported that platelet refractoriness was observed in 25% of the 533 patients who underwent splenectomy.¹¹ The decrease of the platelet refractoriness incidence in patients who underwent splenectomy caused no platelet sequestration, whereas in patients who underwent no splenectomy, up to one third of platelet transfused would be sequestered.¹⁹ The risk of platelet refractoriness in pediatric patients seems to be higher than in adult patients. It is because the blood volume in the spleen of children is relatively higher than those in adults. Moreover, the number of platelets in the spleen is associated with increasing age and weight.¹⁹

Severe bleeding increased the occurrence of risk of platelet refractoriness 3.5 times higher (OR = 3.52) compared to those without severe bleeding, in this study. The risk of platelet refractoriness due to severe bleeding reported in this study was higher than another study conducted in adult patients which reported that RR of platelet refractoriness due to severe bleeding was 2.71.¹¹ Platelet refractoriness in patients with severe bleeding may be caused by activation of coagulation cascade in order

to stop bleeding. Platelets are activated directly at the bleeding site by endothelial damage and indirectly through the clotting cascade. In severe bleeding, various cytokines and tissue factors as well as lipid and phospholipid of tissue are released into the circulation that causes the clotting cascade.^{8,20}

Disseminated intravascular coagulation increased the occurrence of risk of platelet refractoriness 5.4 times higher (OR = 5.41) compared to those without DIC, in this study. This result was similar with another study which reported the RR of platelet refractoriness of children and adult patients with DIC was 5.01.²¹ In DIC, the massive prothrombin activation and the various cytokine release lead to platelet activation directly. In addition, massive endothelial damage in DIC will also activate the clotting cascade that also involves the platelet activation.²² Bacterial surface components such lipopolysaccharides and endotoxins also activate platelets.²³ The stimulation of various platelet activation pathways at the same time can result in the development of DIC as the strongest risk factor for the platelet refractoriness incidence.²⁴

The history of platelet transfusion increased the risk of platelets refractoriness almost 3 times higher (OR = 2.84) compared to patients without the history of platelet transfusion, in this study. This result was higher than another study that reported that the RR of platelets refractoriness in patients having the history of platelet transfusion was 1.56.¹¹ The different results obtained from the two studies might be associated with platelet factors used. Leukoreduction of platelet was used in previous study. It could reduce the platelet refractoriness occurrence from 30% to 5%. The platelet refractoriness occurrence in patients having the history platelet transfusion is thought to be mediated by the formation of the HPA (human platelet antigen) and HLA (human leucocyte antigen) antibodies

through thrombocyte. The HLA formation is 10 times higher than HPA formation. Therefore, the use of leukoreduction of platelet can reduce the occurrence of platelet refractoriness.¹⁰

CONCLUSION

It can be concluded that sepsis, splenomegaly, severe bleeding, DIC, and the history of platelet transfusion are the risk factors of platelet refractoriness in pediatric patients.

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